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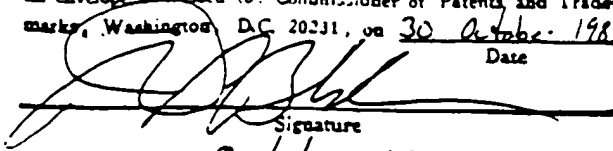
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30 October 1987
Date

Atty Dkt: 2300-0136
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

PAUL A. LUCIW et al.

Serial No.: 773,447

Group Art Unit: 124

Filed: 6 September 1985

Examiner: Moskowitz

For: POLYNUCLEOTIDE SEQUENCES OF VIRUSES ASSOCIATED
WITH LYMPHADENOPATHY SYNDROME AND/OR ACQUIRED
IMMUNE DEFICIENCY SYNDROME

DECLARATION OF DR. NANCY L. HAIGWOOD

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, NANCY L. HAIGWOOD, HEREBY DECLARE:

1. I am employed in the position of Principal Scientist
by Chiron Corporation in Emeryville, California. I hold a
Ph.D. in bacteriology and immunology from the University of
North Carolina, Chapel Hill, and am an expert in immunology,
particularly the immunological properties of recombinant
polypeptides. My qualifications are further summarized on the
attached biographical sketch.

2. It is my expert opinion that one of ordinary skill in
the art of immunology could not have predicted with any degree
of certainty that an immunoassay based on recombinant human
immunodeficiency virus (HIV) polypeptides would perform as
well as an immunoassay employing tissue culture-derived HIV
proteins in the screening of human serum for the presence of
anti-HIV antibodies without actually having performed
experiments to demonstrate the equivalency of the recombinant
polypeptides. I base this opinion on the following facts.

3. As is well known, the AIDS epidemic has posed a serious threat to the safety of the nation's blood banks. It is imperative that immunoassays employed in screening such blood banks have the minimum number of false-negative readings possible. A secondary, yet also important concern, is that the immunoassay not result in unnecessarily high numbers of false positive results. Due to the vast quantities of blood units which must be screened each year, a mere fraction of a percent improvement in these criteria will result in markedly fewer transfusion-caused AIDS cases, while also saving from disposal large quantities of safe blood which is in great demand. Thus, for a recombinant-based immunoassay to be considered a practical alternative to native viral protein-based immunoassays, the recombinant polypeptide-based assay must perform virtually as well as the latter, and not just perform in a "comparable" range.

4. The analyte in the immunoassay of interest is an anti-HIV antibody which has been raised in the patient against the native HIV particle and infected cells. Thus, it must be appreciated that when one proposes to substitute a recombinant polypeptide for the tissue-culture produced HIV protein in an immunoassay, one is suggesting a change at the heart of the immunoassay, i.e., substituting the native substrate (which is virtually the same antigen against which the analyte was raised) with a substrate antigen from a completely heterologous system. It would be entirely believable, therefore, if the anti-HIV antibodies did not bind as well to the recombinant HIV polypeptides as the antibodies bind to the native HIV protein, since the antibodies were actually raised against the latter, not the former.

5. Of particular concern in producing a recombinant polypeptide-based assay for anti-HIV antibodies which leads to unpredictability, is the known heterogeneity of HIV. Prior to the subject invention, it was not clear that the tissue cultures employed to produce the native HIV protein were not

infected by multiple HIV strains. If that were the case, the performance of the native protein-based assays could have been attributed in large part to having a heterogeneous protein substrate which would bind antibodies from a broad cross-section of blood units which had been contaminated by different strains. A recombinant HIV polypeptide is necessarily homogeneous and is clearly derived from a single HIV strain. Thus, one would have no way of knowing until actually carrying out an experiment with a recombinant HIV polypeptide, that a single HIV strain could provide a basis for an effective immunoassay.

6. Another area of unpredictability at the time the present invention was made is the extent to which conformational epitopes unique to native HIV protein were responsible for the performance of immunoassays based on native protein. It has been shown for several viruses (e.g., polio, hepatitis A), that particular monoclonal antibodies to intact virus particles do not bind to disrupted or recombinant virus protein. Thus, it is clear that epitopes to certain conformational aspects of a virus will not be found on recombinantly produced polypeptides. If a conformational epitope unique to the native HIV protein is responsible for the production of a significant proportion of antibodies in HIV infected individuals, then a recombinant polypeptide-based immunoassay would not be expected to be as effective as a native protein-based immunoassay.

7. With regard to specific recombinant polypeptides, there are additional factors which lead to unpredictability of performance of an immunoassay. For example, tissue culture produces very little gp120 env, and it is quite difficult to purify this protein from gp160 env. Thus, immunoassays based on tissue culture-derived protein simply provide no data on which to judge the effectiveness of gp120 env as an antigen. Nor did the art identify prior to the subject invention that p31 pol was at all a useful antigen in an immunoassay.

Furthermore, the gel identification of gp41 env and p25 gag was pure conjecture; i.e., there was no assurance that if a recombinant polypeptide was produced from the DNA sequences encoding the gp41 env or p25 gag regions, that one would have the same polypeptide as had been identified by gel electrophoresis of the native material. Still further, the above discussion has assumed that one is comparing a recombinant polypeptide which is nearly identical in amino acid sequence to a native polypeptide. In the case where recombinant polypeptides differ in amino acid sequence (e.g., fusion proteins) or glycosylation (e.g., bacterial expression), there will be even greater uncertainty regarding the immunological equivalence of a recombinant polypeptide to a native HIV protein.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 26 October, 1987

By: Nancy L. Haigwood
Nancy L. Haigwood

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Publications

Weaver, S.G., N.L. Haigwood, C.A. Hutchison III, and M.H. Edgell (1979). DNA fragments of the Mus musculus beta-globin haplotypes Hbb^d and Hbb^s, Proc. Natl. Acad. Sci. 76, 1385-1389.

Edgell, M.H., S. Weaver, N. Haigwood, and C.A. Hutchison III (1979). Gene Enrichment, in Genetic Engineering, Vol. 1, eds. Setlow and Hollaender (Academic Press, New York), 37-49.

Lautenberger, J.A., C.T. White, N.L. Haigwood, M.H. Edgell, and C.A. Hutchison III (1980). The Recognition Site of Type II Restriction Enzyme BglI is Interrupted, Gene 9, 213.

Jahn, C.L., C.A. Hutchison III, S.J. Phillips, S. Weaver, N.L. Haigwood, C.F. Voliva, and M.H. Edgell (1980). DNA Sequence Organization of the Beta-globin Complex in the BALB/c Mouse, Cell 21, 159-168.

Haigwood, N.L., C.L. Jahn, C.A. Hutchison III, and M.H. Edgell (1981). Locations of three repetitive sequence families found in BALB/c adult beta-globin clones, Nucleic Acids Research 9, 1133-1150.

Edgell, M.H., S. Weaver, C.L. Jahn, R.W. Padgett, S.J. Phillips, C.F. Voliva, M.B. Comer, S.C. Hardies, N.L. Haigwood, C.H. Langley, R.A. Racine, and C.A. Hutchison III (1981). The Mouse Beta Hemoglobin Locus, in Organization and Expression of Globin Genes, eds. G. Stamatoyannopoulos and A.W. Nienhuis (Liss, New York), 69-88.

Burke, R.L., C. Pachl, M. Quiroga, S. Rosenberg, N. Haigwood, O. Nordfang, and M. Ezban (1986). The Functional Domains of Coagulation Factor VIII:C, J. Biol. Chem. 261, 12574-12578.

Haigwood, N., E.-P. Paques, G. Mullenbach, G. Moore, L. DesJardin, A. Tabrizi, S. Brown-Shimer and E.-G. Afting. Improvement of tPA Properties by Means of Site Directed Mutagenesis. (manuscript in preparation)

Current Abstract

Haigwood, N., E.-P. Paques, G. Mullenbach, G. Moore, L. DesJardin, and A. Tabrizi (1987). Improvement of tPA Properties by Means of Site Directed Mutagenesis, XIth 15th Congress, July 1987, Brussels, Belgium.

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Curriculum Vitae

Nancy Logan Haigwood

Date of Birth: November 4, 1951
Place of Birth: Camp Lejeune, North Carolina

Residence: 7050 Sayre Drive
Oakland, CA 94611
(415) 339-3456

Present Position: Principal Scientist, Virology Department
Chiron Corporation
4560 Horton Street
Emeryville, CA 94608
(415) 655-8729

Education: B.S. Zoology, with Honors, 1973
University of North Carolina at Chapel Hill,
Chapel Hill, NC

Ph.D. Bacteriology and Immunology, 1980
School of Medicine, University of North Carolina
at Chapel Hill, Chapel Hill, NC
Dissertation topic:
"The Organization of Repetitive Sequences in Two
Cloned Mouse Beta-Globin Clusters"
Advisors: Drs. Marshall H. Edgell and
Clyde A. Hutchison III

Work Experience:

Chiron Corporation (November 1983 to present)
Principal Scientist, Virology Department

Joined Chiron as a Scientist to develop mammalian cell expression systems. Project leader, human tissue plasminogen activator project, January 1985 to present. Developed tPA mutants with improved biochemical properties in vitro. Promoted to Principal Scientist, Fall, 1985. Developed adenovirus expression vectors for use as substituted nondefective recombinant adenoviruses to express heterologous antigens in vivo. Presently responsible for mammalian cell expression of HIV antigens for use as vaccines in humans.

Genex Corporation (December 1981 to October 1983)

Senior Research Scientist, Molecular Genetics Department

Joined Genex as Assistant to the Chief Scientific Officer in order to gain administrative experience. Responsible for scientific meetings coordination, corporate library, Genex yeast and E. coli strain collection and several other administrative areas.

Transferred to Research Operations in June 1982, and developed site-directed mutagenesis techniques. Constructed mutations in genes to alter protein sequences and to make modifications for expression strategies using oligonucleotide-directed mutagenesis in M13. Participated in proprietary and client sponsored research to improve expression of heterologous genes in E. coli and yeast. Designed, constructed, and demonstrated the utility of a novel expression system for heterologous genes in E. coli. Patent applications pending for discoveries in the area of protein modification and a novel E. coli expression system.

Department of Molecular Biology and Genetics, The Johns Hopkins University
School of Medicine (November 1979 - December 1981) American Cancer Society
Postdoctoral Fellow

Learned animal cell culture and tumor virus biology in the laboratory of Dr. Daniel Nathans. Designed and carried out studies to isolate autonomously replicating sequences from monkey kidney DNA which would function in vivo. Constructed recombinant libraries of monkey DNA in SV40 and plasmid vectors and transfected these libraries into transformed (COS cells) and untransformed monkey kidney cells. Developed a novel assay for replication of transfected DNA in mammalian cells based on the loss of methylation of cloned DNA.

Department of Bacteriology and Immunology, University of North Carolina School of Medicine (1974-1979) University of North Carolina and National Cancer
Postdoctoral Fellow

Learned basic techniques of recombinant DNA and molecular biology in the laboratory of Drs. Marshall Edgell and Clyde Hutchison III. Identified several families of repetitive sequences localized in the mouse beta-globin cluster. Showed that these sequences are repetitive in the genomes of two inbred mouse strains by hybridization to genomic DNA fractionated on RPC-5 or to mouse DNA libraries cloned in bacteriophage lambda. Showed that the C57BL mouse strain has two copies of the adult beta-globin gene, in spite of the apparent expression of only one adult beta-globin gene.

Honors and Fellowships

Irene F. Lee Award, 1973, Outstanding Senior Woman at the University of North Carolina
Order of the Golden Fleece, University of North Carolina Honorary Society
University of North Carolina Graduate Fellowship 1974-75
National Cancer Institute Predoctoral Fellow 1975-78
Mary Poston Award, N.C. Branch of the A.S.M. 1978
American Cancer Society Postdoctoral Fellow 1980-81
Xth Congress Grant, 1987 International Society for Thrombosis and Hemostasis

Professional Societies

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